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IS/ISO 14853 (2005): Determination of ultimate aerobic diodegradability of plastic materials in and aqueous medium - Method by Measurement of biogas production [PCD 12: Plastics]

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प्लास्टिक — जलीय तंत्र में प्लास्टिक सामग्री की अंतिम (अल्टीमेट) ऑक्सी जैव विघटनीयता ज्ञात करना — बायोगैस उत्पादन के मापन द्वारा पद्धति

Indian Standard

PLASTICS — DETERMINATION OF THE ULTIMATE ANAEROBIC BIODEGRADATION OF PLASTIC MATERIALS IN AN AQUEOUS SYSTEM — METHOD BY MEASUREMENT OF BIOGAS PRODUCTION

ICS 83.010.01

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NEW DELHI 110002

January 2009 **Price Group 10**

Plastics Sectional Committee, PCD 12

NATIONAL FOREWORD

This Indian Standard which is identical with ISO 14853 : 2005 'Plastics — Determination of the ultimate anaerobic biodegradation of plastic materials in an aqueous system — Method by measurement of biogas production' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Plastics Sectional Committee and approvai of the Petroleum, Coal and Related Products Division Council.

The text of ISO Standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'.

b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

The technical committee responsible for the preparation of this standard has reviewed the provisions of the following International Standards and has decided that they are acceptable for use in conjunction with this standard:

International Standard	*Title*
ISO 8245 : 1999	Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)
ISO 13641 (all parts)	Water quality — Determination of inhibition of gas production of anaerobic bacteria

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

PLASTICS — DETERMINATION OF THE ULTIMATE ANAEROBIC BIODEGRADATION OF PLASTIC MATERIALS IN AN AQUEOUS SYSTEM — METHOD BY MEASUREMENT OF BIOGAS PRODUCTION

WARNING — Sewage and activated sludge may contain potentially pathogenic organisms. Therefore appropriate precautions should be taken when handling them. Digesting sewage sludge produces flammable gases which present fire and explosion risks. Care should be taken when transporting and storing quantities of digesting sludge. Toxic test chemicals and those whose properties are not known should be handled with care and in accordance with safety instructions. The pressure meter and microsyringes should be handled carefully to avoid needle stick injuries. Contaminated syringe needles should be disposed of in a safe manner.

1 Scope

This International Standard specifies a method for the determination of the ultimate anaerobic biodegradability of plastics by anaerobic microorganisms. The conditions described in this International Standard do not necessarily correspond to the optimum conditions for the maximum degree of biodegradation to occur. The test calls for exposure of the test material to sludge for a period of up to 60 days, which is longer than the normal sludge retention time (25 to 30 days) in anaerobic digesters, though digesters at industrial sites can have much longer retention times.

The method applies to the following materials:

- Natural and/or synthetic polymers, copolymers or mixtures thereof;
- Plastic materials which contain additives such as plasticizers, colorants or other compounds;
- Water-soluble polymers;
- Materials which, under the test conditions, do not inhibit the microorganisms present in the inoculum. Inhibitory effects can be determined using an inhibition control or by another appropriate method (see e.g. ISO 13641). If the test material is inhibitory to the inoculum, a lower test concentration, another inoculum or a pre-exposed inoculum can be used.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 8245, *Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)*

ISO 13641 (all parts), *Water quality — Determination of inhibition of gas production of anaerobic bacteria*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

ultimate anaerobic biodegradation

breakdown of an organic compound by microorganisms in the absence of oxygen to carbon dioxide, methane, water and mineral salts of any other elements present (mineralization) plus new biomass

3.2

primary anaerobic biodegradation

structural change (transformation) of a chemical compound by microorganisms, resulting in the loss of a specific property

3.3

digested sludge

mixture of settled sewage and activated sludge which have been incubated in an anaerobic digester at about 35 °C to reduce the biomass and odour and to improve the dewaterability of the sludge

NOTE Digested sludge contains an association of anaerobic fermentative and methanogenic bacteria producing carbon dioxide and methane.

3.4

concentration of suspended solids in digested sludge

amount of solids obtained by filtration or centrifugation of a known volume of activated sludge and drying at about 105 °C to constant mass

3.5

dissolved organic carbon

DOC

organic carbon in the water phase which cannot be removed by specified phase separation, for example by centrifugation at 40 000 $m \cdot s^{-2}$ for 15 min or by membrane filtration using membranes with pores of 0,2 μm to 0,45 μm diameter

3.6

inorganic carbon

IC

inorganic carbon which is dissolved or dispersed in the aqueous phase of a liquid and is recoverable from the supernatant liquid after the sludge has been allowed to settle

3.7

total dry solids

the amount of solids obtained by taking a known volume of test material or inoculum and drying at about 105 °C to constant mass

3.8

theoretical amount of evolved biogas

Thbiogas

maximum theoretical amount of biogas (CH_4 + CO_2) evolved after complete biodegradation of an organic material under anaerobic conditions, calculated from the molecular formula and expressed as millilitres of biogas evolved per milligram of test material under standard conditions

3.9

theoretical amount of evolved carbon dioxide

$ThCO_2$

maximum theoretical amount of carbon dioxide evolved after complete oxidation of an organic material, calculated from the molecular formula and expressed as milligrams of carbon dioxide per milligram of test material

3.10

theoretical amount of evolved methane

$ThCH_4$

maximum theoretical amount of methane evolved after complete reduction of an organic material, calculated from the molecular formula and expressed as milligrams of methane evolved per milligram of test material

3.11

lag phase

lag period

time, measured in days, from the start of a test until adaptation and/or selection of the degrading microorganisms is achieved and the degree of biodegradation of a chemical compound or organic matter has increased to about 10 % of the maximum level of biodegradation

3.12

plateau phase

time, measured in days, from the end of the biodegradation phase until the end of the test

3.13

biodegradation phase

time, measured in days, from the end of the lag phase of a test until about 90 % of the maximum level of biodegradation has been reached

3.14

maximum level of biodegradation

degree of biodegradation, measured in percent, of a chemical compound or organic matter in a test, above which no further biodegradation takes place during the test

4 Principle

The biodegradability of a plastic material is determined using anaerobic conditions in an aqueous system. Test material with a concentration of 20 mg/l to 200 mg/l organic carbon (OC) is incubated at (35 ± 2) °C in sealed vessels together with digested sludge for a period normally not exceeding 60 days. Before use, the digested sludge is washed so that it contains very low amounts of inorganic carbon (IC) and diluted to 1 g/l to 3 g/l total solids concentration. The increase in headspace pressure or the volumetric increase (depending on the method used for measuring biogas evolution) in the test vessels resulting from the production of carbon dioxide (CO_2) and methane (CH_4) is measured. A considerable amount of CO_2 will be dissolved in water or transformed to bicarbonate or carbonate under the conditions of the test. This inorganic carbon (IC) is measured at the end of the test. The amount of microbiologically produced biogas carbon is calculated from the net biogas production and the net IC formation in excess of blank values. The percentage biodegradation is calculated from the total amount of carbon transformed to biogas and IC and the measured or calculated amount of carbon added as test material. The course of biodegradation can be followed by making intermediate measurements of biogas production. As additional information, the primary biodegradability can be determined by specific analyses at the beginning and end of the test.

This test method is designed to determine the biodegradability of plastic materials under anaerobic conditions. Optionally, the assessment of the recovery rate may also be of interest (see Annex G).

5 Reagents and materials

5.1 Distilled or deionized water, free of toxic substances, containing less than 2 mg/l of DOC.

5.2 Test medium, prepared using only reagents of recognized analytical grade.

Prepare the test medium to contain the following constituents in the stated amounts:

Anhydrous potassium dihydrogen phosphate	KH_2PO_4	0,27 g

Disodium hydrogen phosphate dodecahydrate	$Na_2HPO_4 \cdot 12H_2O$	1,12 g
Ammonium chloride	NH_4Cl	0,53 g
Calcium chloride dihydrate	$CaCl_2 \cdot 2H_2O$	0,075 g
Magnesium chloride hexahydrate	$MgCl_2 \cdot 6H_2O$	0,10 g
Iron(II) chloride tetrahydrate	$FeCl_2 \cdot 4H_2O$	0,02 g
Resazurin (oxygen indicator)		0,001 g
Disodium sulfide (see note)	$Na_2S \cdot 9H_2O$	0,1 g
Stock solution of trace elements (optional)		10 ml
Stock solutions of vitamins (optional)	Vitamin solution No. 1	0,5 ml
	Vitamin solution No. 2	0,5 ml
Add water (5.1) (oxygen-free) to		1 l

Adjust the pH of the medium with dilute mineral acid or alkali, if necessary, to 7 ± 0,2.

To ensure oxygen-free conditions, purge the water with nitrogen for about 20 min immediately before use.

Use freshly prepared sodium sulfide, or wash and dry it before use, to ensure sufficient reductive capacity. In order to ensure strictly anaerobic conditions, it is recommended that a small amount of sodium dithionite be added to the medium after it has been prepared until it becomes colourless. Do not use more than 10 mg/l, because higher concentrations may produce inhibitory effects.

5.3 Trace-element solution (optional).

It is recommended that the test medium be supplemented with the following trace elements to improve the anaerobic degradation process, especially if low inoculum concentrations are used:

Manganese chloride tetrahydrate	$MnCl_2 \cdot 4H_2O$	0,05 g
Boric acid	H_3BO_3	0,005 g
Zinc chloride	$ZnCl_2$	0,005 g
Copper chloride	$CuCl_2$	0,003 g
Disodium molybdate dihydrate	$Na_2MoO_4 \cdot 2H_2O$	0,001 g
Cobalt chloride hexahydrate	$CoCl_2 \cdot 6H_2O$	0,1 g
Nickel chloride hexahydrate	$NiCl_2 \cdot 6H_2O$	0,01 g
Disodium selenite	Na_2SeO_3	0,005 g
Disodium tungstate	$Na_2WO_4 \cdot 2H_2O$	0,002 g
Add water (5.1) (oxygen free) to		1 l

Use 10 ml of trace-element solution per litre of test medium.

5.4 Vitamin solutions (optional)

5.4.1 Vitamin solution No. 1

4-Aminobenzoic acid	40 mg
D-Biotin	10 mg
Dissolve in hot water (5.1)	500 ml
Allow to cool and add:	
D-Pantothenic acid, calcium salt	50 mg
Pyridoxamine dihydrochloride	150 mg
Thiamine dichloride	100 mg

Filter the solution through a membrane filter (pore size 0,45 µm) that neither adsorbs nor releases organic carbon in significant amounts, and store in the dark at 4 °C.

Use 0,5 ml of vitamin solution per litre of test medium.

5.4.2 Vitamin solution No. 2

Cyanocobalamin (vitamin B12)	10 mg
Dissolve in water (5.1)	100 ml

Filter the solution through a membrane filter (pore size 0,45 µm) that neither adsorbs nor releases organic carbon in significant amounts, and store in the dark at 4 °C.

Use 0,5 ml of vitamin solution per litre of test medium.

5.5 Barrier solution

NaCl	200 g
Dissolve in water (5.1)	1 000 ml
Acidify with citric acid	5 g

Add a pH-indicator such as bromophenol blue or methyl orange in order to be able to verify that the solution remains acid during the test.

5.6 Test material

The test material is usually added directly as solid to give a concentration of 20 mg/l to 200 mg/l organic carbon. The test material (plastic) should be used in powdered form if possible.

The biodegradability of plastic materials which are not inhibitory to microorganisms can be determined using concentrations higher than 200 mg/l organic carbon. In this case, ensure that the buffer capacity and mineral-salt content of the medium are sufficient.

5.7 Reference material

Use a well-defined anaerobically biodegradable polymer, e.g. poly-β-hydroxybutyrate, cellulose or poly(ethylene glycol) 400 as a reference material. If possible, the form, size, solubility and concentration of the reference material should be comparable to that of the test material.

Prepare the reference material in the same way as the test material.

5.8 Inhibition control (optional).

Add both the test material and the reference material to a vessel containing test medium (5.2) to give the concentrations specified in 5.6 and 5.7, respectively.

6 Apparatus

6.1 Laboratory equipment

Required is usual laboratory equipment, plus the following:

6.1.1 Incubator or water or sand bath, thermostatically controlled at (35 ± 2) °C.

6.1.2 Carbon analyser, suitable for the direct determination of inorganic carbon in the range 1 mg/l to 200 mg/l IC

6.2 Apparatus for use when biogas is measured by a manometric method

6.2.1 Pressure-resistant glass test vessels, nominal size 0,1 litre to 1 litre, each fitted with a gastight septum capable of withstanding about 2 000 hPa (for an example, see Annex A). The headspace volume shall be about 10 % to 30 % of the total volume. If gas is released at regular intervals, about 10 % headspace volume is adequate, but if gas is released only at the end of the test, 30 % is more appropriate.

NOTE From a practical point of view, the use of serum bottles sealed with butyl rubber serum caps and crimped aluminium rings is recommended.

6.2.2 Pressure-measuring device, e.g. a manometer connected to a suitable syringe needle, with a gastight three-way valve to facilitate the release of excess pressure. Use and calibrate the device in accordance with the manufacturer's instructions.

NOTE It is necessary to keep the internal volume of the tubing and the valve as low as possible so that errors introduced by neglecting the volume of the device are not significant.

6.3 Apparatus for use when biogas is measured by a volumetric method

6.3.1 Glass test vessels (e.g. conical flasks or bottles), nominal size 0,1 litre to 1 litre, preferably 300 ml for every 250 ml of medium. If foaming is not expected to occur, a headspace volume of 10 % to 20 % is recommended. The vessels shall be equipped with a septum for gas sampling (see Annex B) and shall be connected via gastight tubing to a graduated glass gas-collection tube which is filled with acidified salt solution (barrier solution 5.5). This graduated glass tube shall be connected to an expansion tank which can be moved up and down to bring the surface of the acidified solution in the expansion tank to the same level as that in the gas-collection tube.

7 Procedure

7.1 General

Carry out the following initial operations using techniques which will ensure that the digested sludge comes into contact with oxygen as little as practicable, e.g. work in a glove-box in an atmosphere of nitrogen or purge the test vessels with nitrogen.

7.2 Digested sludge

Collect digested sludge from a digester at a sewage treatment plant treating predominantly domestic sewage. Be sure to collect active sludge. Use wide-necked bottles made of high-density polyethylene or a similar material which can expand. Glass is not recommended for safety reasons. Fill the bottles to within 1 cm of the top and seal. After transport to the laboratory, use directly or place in a laboratory-scale digester. Release excess biogas.

Alternatively, use a laboratory-grown anaerobic sludge as a source of the inoculum.

Consider pre-incubation of the sludge to reduce background gas production and to decrease the influence of the blanks. Allow the sludge to digest, without the addition of any nutrients or substrates, at (35 ± 2) °C for up to 7 days.

It has been shown that pre-incubation for about 5 days gives an optimum decrease in gas production by the blank without an unacceptable increase in either lag period or incubation period during the test. For test materials which are expected to be poorly biodegradable, consider pre-incubating the sludge with the test material to get a better adapted inoculum. In such a case, add test material with a concentration of 5 mg/l to 20 mg/l OC to the digested sludge. Wash the pre-incubated sludge carefully before use. Indicate in the test report that pre-incubation was carried out.

7.3 Preparation of the inoculum

Wash the sludge just prior to use to reduce the IC content to less than 20 mg/l in the final test suspension. If the IC has not been sufficiently lowered, wash the sludge an additional two times. Finally, suspend the sludge in the requisite volume of test medium (5.2) and determine the concentration of total solids (see 3.7). The final concentration of total solids in the test vessels shall be in the range 1 g/l to 3 g/l. Conduct the above operations in such a way that the sludge has minimal contact with oxygen (e.g. use a nitrogen atmosphere).

7.4 Preparation of test suspensions and controls

At least three test vessels F_T shall be prepared for the test material, at least three for the blanks F_B and at least one vessel F_P for the positive control (reference material). One or more vessels F_I may optionally be prepared for each test material as an inhibition control (see Table 1). The same blanks and controls can be used for several different test materials which are being tested together. Into all the vessels, introduce aliquots of the diluted inoculum prepared in 7.3 so that the concentration of total solids is the same in all the vessels — between 1 g/l and 3 g/l. Add the test material (5.6) and the reference material (5.7) to the appropriate vessels. The OC concentration in the test suspensions shall normally be 100 mg/l. In the case of toxic test materials, it may be reduced to 20 mg/l OC or even less if only the primary biodegradability is to be determined with specific analyses.

NOTE Using lower test concentrations may result in a greater scatter of the test results.

In the case of the blank vessels, add equivalent amounts of oxygen-free water (5.1) instead of the test material. An extra (replicate) test vessel containing test suspension may also be prepared for analyses, carried out at the beginning of the test, to determine the pH and, if required, the total solids and IC.

Adjust the pH to 7 ± 0,2, if necessary, with small amounts of dilute mineral acid or alkali. Add the same amount of neutralizing agent to all the test vessels. If the primary degradability is to be measured, take a suitable sample from the extra test vessel and measure the test material concentration using a suitable method. Place magnetic stirrer bars in the vessels if the test suspensions are to be stirred (optional). Ensure that the total volume of liquid V_L and the volume of the headspace V_H are the same in all vessels (see 6.2.1). Note V_L and V_H (see Clause 8). If necessary, add additional oxygen-free test medium (5.2). Seal each vessel with a gastight septum and put them into the incubator (6.1.1).

Table 1 — Scheme of test and control assays

Vessel	Test material	Reference material (biodegradable)	Inoculum
F_{T1} Test	+		+
F_{T2} Test	+		+
F_{T3} Test	+		+
F_{B1} Blank			+
F_{B2} Blank			+
F_{B3} Blank			+
F_P Positive control		+	+
Extra replicate for analysis at beginning of test	+		+
F_I Inhibition control (optional)	+	+	+

7.5 Incubation and gas measurement

7.5.1 General

Incubation shall take place in sealed vessels at a constant temperature of (35 ± 2) °C, a normal temperature for an anaerobic digester, in the absence of oxygen, initially in an atmosphere of pure nitrogen.

7.5.2 Gas measurement using a manometer (see Annex A)

Incubate the prepared vessels at (35 ± 2) °C for about 1 h to allow equilibration, and vent excess gas to the atmosphere, for example by shaking each vessel in turn, inserting the needle of the manometer through the seal and opening the valve until the manometer reads zero. If at this stage, or when making intermediate measurements, the headspace pressure is less than atmospheric, introduce nitrogen gas to re-establish atmospheric pressure. Close the valve and continue to incubate in the dark, ensuring that all parts of the vessels are maintained at the incubation temperature.

Observe the vessels after incubation for 24 h to 48 h. Reject vessels if their contents show a distinct pink coloration in the supernatant liquid. This is due to a change in colour of the resazurin, indicating the presence of oxygen. While small amounts of oxygen can be tolerated in the system, higher concentrations can seriously inhibit the course of anaerobic biodegradation.

Carefully mix the contents of each vessel by stirring or shaking for a few minutes at least two or three times per week and before each pressure measurement. Measure the gas pressure, for example by inserting, through the septum, the syringe needle connected to the manometer. Record the pressure in hectopascals.

Shaking resuspends the inoculum and ensures gas equilibrium. While measuring pressure, maintain the gas in the headspace at the incubation temperature. Take care to prevent water entering the syringe needle. Should this occur, dry the wetted parts and fit a new needle.

Either measure the gas pressure in the vessels weekly, venting excess gas to the atmosphere, or measure the pressure only at the end of the test to detect the total amount of biogas produced. It is strongly recommended, however, that intermediate readings of gas pressure be made, since the pressure increase provides guidance as to when the test may be terminated and allows the kinetics to be followed.

7.5.3 Gas measurement using a volumetric device (see Annex B)

The biogas produced can be collected in a graduated glass tube separated from the atmosphere by a barrier solution in such a way that the pressure remains nearly constant (except for atmospheric pressure changes) during the test. After incubation of the prepared vessels at (35 ± 2) °C for about 1 h, vent excess gas to the atmosphere, for example by shaking each vessel in turn, inserting a syringe needle through the septum seal and allowing gas to escape until the surface of the barrier solution in the gas-collection tube reaches zero. Make sure that the surface of the barrier solution in the expansion tank is at the same level as in the gas-collection tube. Remove the syringe needle and continue to incubate in the dark, ensuring that all parts of all the vessels are maintained at the incubation temperature.

Readings of the gas volume can be made directly from the gas-collection tube. Before taking a reading, bring the surface of the liquid in the expansion tank to the same level as the surface of the liquid in the collection tube so that the gas volume is read at atmospheric pressure (see Annex B for operating instructions). Make a sufficient number of measurements of gas volume, pressure and temperature (normally every day) to determine the rate of gas production. More frequent readings in the early stages may be required, with less frequent readings needed as time progresses.

7.6 Test duration

The normal test duration is 60 days. The test may be terminated earlier if the biodegradation curve obtained from the pressure or volume measurements has reached a plateau phase (see 3.12). If, at the end of the normal incubation period, an obvious plateau phase has not been reached, the test can be extended till such time as a plateau phase is reached. However, the test duration shall not exceed 90 days.

7.7 Measurement of inorganic carbon

At the end of the test, after the last measurement of gas pressure or increase in gas volume, allow the sludge to settle, open each vessel and immediately determine the concentration of inorganic carbon (IC) (in mg/l) in the supernatant liquid. The supernatant liquid shall not be centrifuged or filtered at this stage (see Note). After IC measurement, record the pH. Carry out similar measurements on the blanks, the reference material and any optional controls.

NOTE Centrifugation or filtration would result in an unacceptable loss of dissolved carbon dioxide. If the sample of supernatant liquid cannot be analysed immediately, it may be stored in a suitable sealed vial, without headspace, at about 4 °C for up to 2 days.

In some cases, especially if the same blanks or controls are used for several different test materials, give consideration to measuring intermediate IC concentrations in the test and control vessels. In this case, use the following procedure.

After measuring the gas pressure or the volume increase without releasing excess gas, take an aliquot of the supernatant liquid which is as small as possible with a syringe through the septum without opening the vessel and determine the IC in the sample. After having taken the sample, excess gas may be vented from the vessel (see 7.5).

Note that even a small decrease in the volume of the supernatant liquid (e.g. about 1 %) can produce a significant increase in the volume of the headspace. Correct Equation (3) in 8.2 by increasing V_H if necessary.

7.8 Specific analyses

If primary anaerobic biodegradability is to be determined, perform specific analyses of the test material in the replicate flask (see 7.4) at the beginning and the end of the test. If this is done, note that the volume of the headspace (V_H) and the liquid (V_L) will be changed and this will have to be taken into consideration when calculating the results.

8 Calculation and expression of results

8.1 Amount of carbon in headspace

1 mol of methane and 1 mol of carbon dioxide each contain 12 g of carbon. Calculate the carbon content of a given volume of evolved gas using Equation (1):

$$m = 12\,000 \cdot n \tag{1}$$

where

m	is the mass of carbon, in milligrams, in a given volume of evolved gas;
12 000	is the relative atomic mass of carbon, in milligrams;
n	is the number of moles of gas.

8.2 Calculation of amount of carbon in headspace when manometric measurement method used

Calculate n from the gas law as given by Equation (2):

$$n = \frac{pV}{RT} \tag{2}$$

where

n	is the number of moles of gas;
p	is the pressure, in pascals, of the gas;
V	is the volume, in cubic metres, of the gas;
R	is the molar gas constant (8,314 J/mol·K);
T	is the incubation temperature, in kelvins.

Calculate the net mass of carbon (after subtracting the mean blank values) produced as gas in the headspace from the test material using Equation (3):

$$m_\text{h} = \frac{12\,000 \times 0{,}1 \times (\Delta p \times V_\text{H})}{RT} \tag{3}$$

where

m_h	is the net mass, in milligrams, of carbon produced as gas in the headspace;
V_H	is the volume of the headspace, in litres;
Δp	is the mean difference between the initial and final pressures, in hectopascals, in each test vessel minus that for the blank vessels;
0,1	is a conversion factor for both hectopascals to pascals and litres to cubic metres;
12 000	is the relative atomic mass of carbon, in milligrams.

For the normal incubation temperature of 35 °C (308°K), Equation (4) can be used:

$$m_\text{h} = 0{,}468 \times (\Delta p \cdot V_\text{H}) \tag{4}$$

If appropriate, the course of the biodegradation can be followed by plotting the cumulative pressure increase Δp, in hectopascals, against time. From this curve, the lag phase (see 3.11) can be identified and recorded in days (for an example, see Annex C).

8.3 Calculation of amount of carbon in headspace when volumetric measurement method used

When the amount of biogas produced is measured volumetrically, such as by a liquid displacement system, use Equations (2.1), (3.1) and (4.1) to calculate n and m_h:

$$n = \frac{(p - p_w) \cdot V}{RT} \tag{2.1}$$

$$m_h = \frac{12\,000 \times 0{,}1 \times (p - p_w) \times \Delta V_H}{RT} \tag{3.1}$$

$$m_h = 0{,}468 \times (p - 5{,}62) \times \Delta V_H \tag{4.1}$$

where

p_w is the pressure, in hectopascals, of water vapour at the incubation temperature (see Annex E for a table of water vapour pressures);

p is the atmospheric pressure, in hectopascals;

5,62 is the water vapour pressure, in hectopascals, at 35 °C;

ΔV_H is the mean difference between the initial and final volumes, in litres, of the headspace in each test vessel minus that for the blank vessels;

the other symbols are as defined for Equations (2), (3) and (4).

8.4 Amount of inorganic carbon in the liquid

Calculate the carbon content of the liquid in the test vessels using Equation (5):

$$m_L = \rho_{IC,net} \cdot V_L \tag{5}$$

where

m_L is the mass, in milligrams, of inorganic carbon in the liquid;

$\rho_{IC,net}$ is the mean concentration of inorganic carbon, in milligrams per litre, in the test vessels minus that in the blank vessels at the end of the test;

V_L is the volume, in litres, of liquid in the vessel.

8.5 Total amount of carbon converted to gas

Calculate the total amount of carbon converted to gas using Equation (6):

$$m_t = m_h + m_L \tag{6}$$

where

m_t is the total mass, in milligrams, of carbon converted to gas;

m_h and m_L are as defined in 8.2 and 8.4.

8.6 Amount of carbon in test material

Calculate, from the concentration of carbon in the test material, the mass of carbon in the test material in each vessel using Equation (7):

$$m_{\mathrm{v}} = \rho_{\mathrm{C,v}} \cdot V_{\mathrm{L}} \tag{7}$$

where

m_{v} is the mass, in milligrams, of carbon in the test material;

$\rho_{\mathrm{C,v}}$ is the concentration, in milligrams per litre, of carbon in the test material;

V_{L} is the volume, in litres, of liquid in the vessel.

8.7 Calculation of percentage biodegradation

Calculate the percentage biodegradation from the headspace gas measurements using Equation (8) and the total percentage biodegradation using Equation (9):

$$D_{\mathrm{h}} = \frac{m_{\mathrm{h}} \times 100}{m_{\mathrm{v}}} \tag{8}$$

$$D_{\mathrm{t}} = \frac{m_{\mathrm{t}} \times 100}{m_{\mathrm{v}}} \tag{9}$$

where

D_{h} is the percentage biodegradation from the headspace gas measurements;

D_{t} is the total percentage biodegradation;

m_{h}, m_{v} and m_{t} are as defined in 8.2, 8.6 and 8.5, respectively.

9 Validity of results

9.1 Maintenance of anaerobic conditions

Use only data from vessels which contained no oxygen, i.e. which showed no pink coloration. Contamination by oxygen can be minimized by the use of correct anaerobic handling techniques.

9.2 Inhibition of degradation

Gas production in the vessel containing both test material and reference material shall be at least equal to that in the vessel with reference material only. If it is not, then this indicates that gas production is being inhibited. In the latter case, repeat the test using a lower concentration of test material, but not less than 20 mg/l OC (see 7.4).

9.3 Validity of the test

Consider the test to be valid if the reference material has a plateau phase that represents >70 % biodegradation (see bibliographic reference [4]). If the pH at the end of the test is outside the range 7 ± 1 and insufficient biodegradation has taken place, the test shall be repeated using a test medium (5.2) with higher buffer capacity. If less than 70 % biodegradation is observed with the positive reference (on the basis of measurements of the amount of biogas in the headspace and IC in the liquid), the test shall be regarded as invalid and shall be repeated with fresh inoculum.

10 Test report

The test report shall contain at least the following information:

a) a reference to this International Standard;

b) all information necessary to identify the test and reference materials, including their TOC, ThC, $ThCO_2$, $ThCH_4$, chemical composition and formula (if known), shape, form and amount/concentration in the samples tested;

c) the concentration of the test material in the test flasks;

d) details of the way in which the amount of biogas produced was measured (e.g. type of pressure-measuring device or volume-measuring system used) and of the carbon analyser used to measure IC;

e) all the results obtained during the test from the test vessels, blanks, positive controls and inhibition controls, if used (e.g. pressure in hPa, volume in ml and IC in mg/l), in tabular form (examples of data sheets are given in Annex D), plus the statistical treatment of the results;

f) information on the inoculum, including source, date of collection and use, storage, handling, any adaptation to the test material and any other pre-incubation;

g) the incubation temperature;

h) the volume of the liquid (V_L) and the headspace (V_H) in the vessels;

i) the pH and IC of the test suspensions at the beginning and the end of the test;

j) the concentration of the test material at the beginning and the end of the test if specific analyses were carried out;

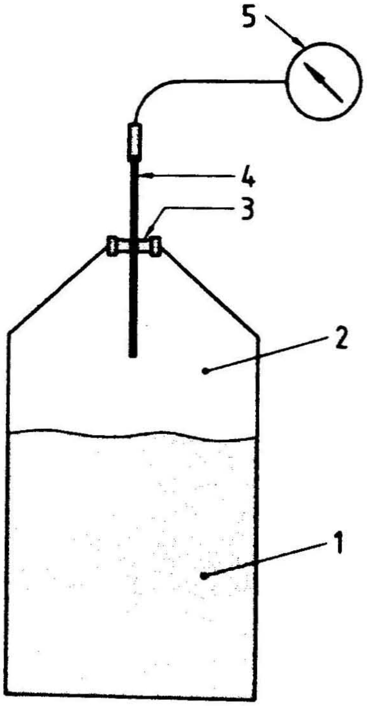
k) the biodegradation curve plotted from the headspace gas measurements;

l) the percentage biodegradability of the test material and the reference material (mean values), the final test result being indicated as a 10 % range (e.g. 20 % to 30 %);

m) the duration of the lag phase and the degradation phase and the duration of the test.

Annex A
(informative)

Example of apparatus for determining the amount of biogas produced by measuring the increase in gas pressure



Key

1 test suspension (volume V_L)

2 headspace (volume V_H)

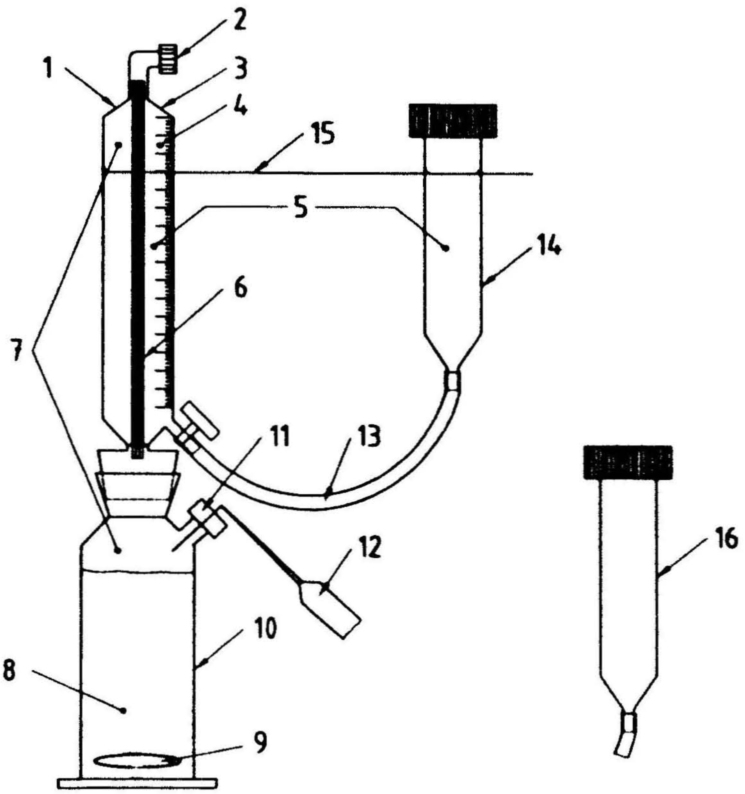
3 gastight seal

4 syringe needle

5 manometer

Annex B
(informative)

Example of apparatus for determining volumetrically the amount of biogas produced



Key

1 graduated glass gas-collection tube (capacity 150 ml to 200 ml)
2 gas-sampling port
3 minimum headspace 5 ml
4 headspace gas
5 barrier solution
6 glass tube allowing gas evolved to pass into collection tube
7 headspace (volume V_H)
8 test suspension (volume V_L)
9 magnetic stirrer
10 test vessel (capacity 300 ml to 350 ml)
11 gas- or liquid-sampling port
12 purging probe
13 flexible connecting tubing
14 reservoir (open to atmosphere) of barrier solution, adjustable in height
15 position 0 (measurement position)
16 position 1 (for checking leaktightness)

Figure B.1 — Apparatus for determining volumetrically the amount of biogas produced

B.1 Purging with N_2 or Ar

In order to ensure an oxygen-free atmosphere within the system, connect the test vessel to the gas-collection tube while passing a stream of oxygen-free gas over the surface of the test suspension. This can be done by means of a purging probe, introduced through the septum in the sampling port of the test vessel, as follows. Close the gas-sampling port of the gas-collection tube so that the gas which evolves will push the barrier solution round into the reservoir tank. Then open the gas-sampling port until the gas-collection tube is refilled with barrier solution (do not overfill the gas-collection tube, but maintain a minimum headspace of about 5 ml). Repeat the purging procedure two or three times. Then remove the purging probe and check the gastightness as described below.

B.2 Checking gastightness

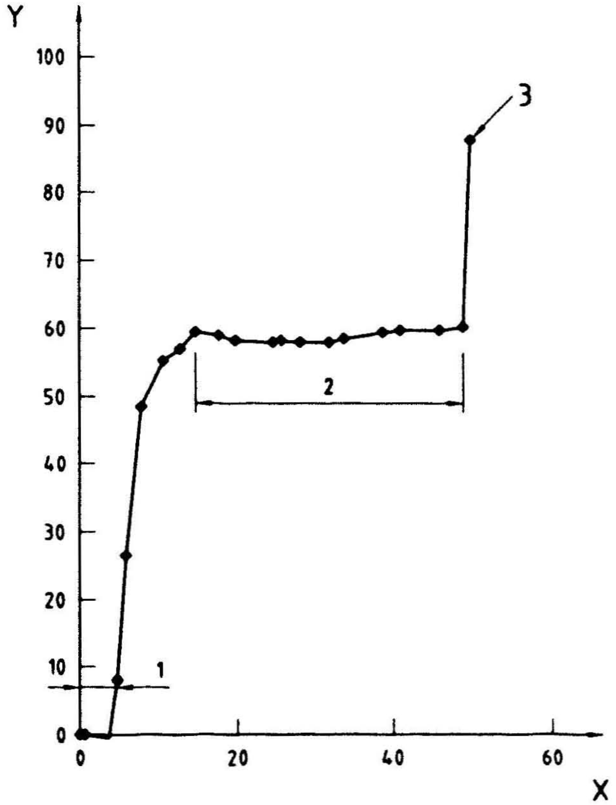
With the gas-collection tube connected to the test vessel, fill the gas-collection tube with barrier solution and close the gas- and liquid-sampling port. Then move the reservoir (connected by flexible tubing to the gas-collection tube) to position 1 to create a low pressure in the gas-collection tube. If the system is not gastight, the barrier solution will flow from the gas-collection tube into the reservoir. If the system is gastight, raise the reservoir to position 0. This is the position at which the surface of the barrier solution in the reservoir and in the gas-collection tube are at the same level, so that the volume of biogas produced can be measured at atmospheric pressure.

Annex H
(informative)

Example of a workflow scheme

Annex C
(informative)

Example of a biodegradation curve



Key

X time (days)
Y degree of biodegradation (%)
1 lag phase
2 plateau phase
3 + IC

Annex D
(informative)

Examples of data sheets for anaerobic biodegradability tests

This annex gives examples of data sheets for use when pressure (manometric) measurements are made and when volume measurements are made.

Anaerobic biodegradability test — Data sheet for PRESSURE measurements

Laboratory: ____________ Test material: ____________ Test No.: ________

Test temperature: _____(°C) Headspace volume (V_H): _______ (litres) Volume of liquid (V_L): ___ (litres) Carbon in test material $\rho_{C,v}$: _______ (mg/l) m_v [a]: _____(mg)

Day	p_1 (test) hPa	p_2 (test) hPa	p_3 (test) hPa	p (test) mean hPa	p_4 (blank) hPa	p_5 (blank) hPa	p_6 (blank) hPa	p (blank) mean hPa	p (net) test mean minus blank mean hPa	Δp (net) cumulative hPa	m_h headspace C [b] mg	D_h degree of biodegradation [c] %
	$\rho_{IC,1}$ test mg/l	$\rho_{IC,2}$ test mg/l	$\rho_{IC,3}$ test mg/l	ρ_{IC} test mean mg/l	$\rho_{IC,4}$ blank mg/l	$\rho_{IC,5}$ blank mg/l	$\rho_{IC,6}$ blank mg/l	ρ_{IC} blank mean mg/l	$\rho_{IC,net}$ test mean minus blank mean mg/l	m_L C in liquid [d] mg	m_t total C [e] mg	D_t total degree of biodegradation [f] %
IC (at end)												
pH (at end)												

[a] Carbon in test vessel, m_v (mg) $m_v = \rho_{C,v} \cdot V_L$

[b] Carbon in headspace m_h (mg) at normal incubation temperature (35 °C) $m_h = 0.468 (\Delta p \cdot V_H)$

[c] Degree of biodegradation D_h calculated from headspace gas measurements $D_h = \frac{m_h \cdot 100}{m_v}$

[d] Carbon in liquid, m_L (mg) $m_L = \rho_{IC,net} \cdot V_L$

[e] Total gasified carbon, m_t (mg) $m_t = m_h + m_L$

[f] Total degree of biodegradation, D_t (%) $D_t = \frac{m_t \cdot 100}{m_v}$

Anaerobic biodegradability test — Data sheet for VOLUME measurements

Laboratory ______________________ Test material ______________________ Test No ________

Test temperature _____(°C) Headspace volume (V_H) ________ (litres) Volume of liquid (V_L) ___ (litres) Carbon in test material $\rho_{C,v}$ ________ (mg/l) m_v [a] ____ (mg)

Day	Atmos. press hPa	V_1 (test) Biogas in ml	V_2 (test) Biogas in ml	V_3 (test) Biogas in ml	V (test) mean Biogas in ml	V_4 (blank) Biogas in ml	V_5 (blank) Biogas in ml	V_6 (blank) Biogas in ml	V (blank) mean Biogas in ml	V (net) test mean minus blank mean Biogas in ml	ΔV (net) cumulative Biogas in ml	m_h headspace C [b] mg	D_h degree of biodegradation [c] %
		$\rho_{IC,1}$ test mg/l	$\rho_{IC,2}$ test mg/l	$\rho_{IC,3}$ test mg/l	ρ_{IC} test mean mg/l	$\rho_{IC,4}$ blank mg/l	$\rho_{IC,5}$ blank mg/l	$\rho_{IC,6}$ blank mg/l	ρ_{IC} blank mean mg/l	$\rho_{IC,net}$ test mean minus blank mean mg/l	m_L C in liquid [d] mg	m_t total C [e] mg	D_t total degree of biodegradation [f] %
IC (at end)													
pH (at end)													

[a] Carbon in test vessel, m_v (mg): $m_v = \rho_{C,v} \cdot V_L$

[b] Carbon in headspace m_h (mg) at normal incubation temperature (35 °C): $m_h = 0.468 \times (p - p_w) \times \Delta V_h$ where p is the atmospheric pressure and p_w is the water vapour pressure

[c] Degree of biodegradation D_h calculated from headspace gas measurements: $D_h = \frac{m_h \cdot 100}{m_v}$

[d] Carbon in liquid, m_L (mg): $m_L = \rho_{IC,net} \cdot V_L$

[e] Total gasified carbon, m_t (mg): $m_t = m_h + m_L$

[f] Total degree of biodegradation, D_t (%): $D_t = \frac{m_t \cdot 100}{m_v}$

Annex E
(informative)

Table of water vapour pressures at various temperatures

T °C	p kPa
20	2,338 8
21	2,487 7
22	2,644 7
23	2,810 4
24	2,985 0
25	3,169 0
26	3,362 9
27	3,567 0
28	3,781 8
29	4,007 8
30	4,245 5

T °C	p kPa
31	4,495 3
32	4,757 8
33	5,033 5
34	5,322 9
35	**5,626 7**
36	5,945 3
37	6,279 5
38	6,629 8
39	6,996 9
40	7,381 4
41	7,784 0

Data taken from *CRC Handbook of Chemistry and Physics* [6]

Annex F
(informative)

Calculation of theoretical carbon dioxide ($ThCO_2$) and theoretical methane ($ThCH_4$) production

The maximum theoretical amounts of CO_2 and CH_4 produced are given by Equation (F.1) according to Buswell and Mueller [2]:

$$C_cH_hO_o + \left(c - \frac{h}{4} - \frac{o}{2}\right)H_2O \rightarrow \left(\frac{c}{2} + \frac{h}{8} - \frac{o}{4}\right)CH_4 + \left(\frac{c}{2} - \frac{h}{8} + \frac{o}{4}\right)CO_2 \qquad (F.1)$$

The extended Buswell and Mueller equation for materials containing nitrogen and/or sulfur is given by Equation (F.2):

$$\begin{aligned} C_cH_hO_oN_nS_s + \left(c - \frac{h}{4} - \frac{o}{2} + \frac{3n}{4} + \frac{s}{2}\right)H_2O &\rightarrow \left(\frac{c}{2} + \frac{h}{8} - \frac{o}{4} - \frac{3n}{8} - \frac{s}{4}\right)CH_4 \\ &+ \left(\frac{c}{2} - \frac{h}{8} + \frac{o}{4} + \frac{3n}{8} + \frac{s}{4}\right)CO_2 + nNH_3 + sH_2S \end{aligned} \qquad (F.2)$$

In the presence of oxidizing agents such as Fe_2O_3 and SO_4^{2-}, the Buswell and Mueller equation is not applicable.

Annex G
(informative)

Example of determination of recovery rate

G.1 Principle

Plastic materials are normally of more complex composition compared to materials with low molecular masses Measurement of the biogas evolution may be not sufficient in some circumstances to characterize and quantify their biodegradability. During biodegradation, new biomass is produced by the microorganisms, and part of the carbon in the test material is transformed into biomass and not converted biochemically into biogas Thus test parameters such as CO_2 production, IC and CH_4 production will often not reach 100 % of the theoretical value, even in the case of complete biodegradation of a test material. An insufficient degree of biodegradation could erroneously be deduced from such results. Due to the low yield coefficient of the anaerobic consortia concerned, the amount of new biomass will be less compared to aerobic cultures. Nevertheless, it may be valuable in some circumstances to estimate the amount of the biomass at the beginning and the end of the test. It should, however, be taken into account that the methods for the measurement and calculation of the biomass in anaerobic systems often lead to unsatisfactory results. The determination of the recovery rate, as described in this annex, may be helpful in such cases to confirm complete biodegradability.

Determination of the recovery rate is based on the amounts of carbon determined by the following measurements carbon evolved as carbon dioxide gas, water-soluble inorganic carbon (IC) and methane evolved as gas, carbon in the form of new biomass produced, water-soluble organic metabolites produced in the degradation process and determined as DOC and the carbon content of the residual undegraded polymeric material The sum of this carbon is compared with the amount of organic carbon in the test material introduced into the test system.

G.2 Test procedure

G.2.1 Determine the amount of biogas (CO_2 and CH_4).

G.2.2 Determine the amount of inorganic carbon in the aqueous phase.

G.2.3 Determine the DOC in the filtered test suspensions or in the supernatant liquid using e.g. ISO 8245 and calculate the increase in organic carbon. Try to identify, if possible, the materials forming the DOC in order to confirm that water-soluble metabolites have been produced.

It is recommended that the DOC be measured at the beginning and the end of the test If water-soluble polymer components are present, the question will have to be examined as to whether the DOC can be counted as the biodegraded part of the sample or not. It is recommended that any high DOC values obtained at the end of the test be scrutinized closely, as they could be an indication that metabolites or polymer components are present which could accumulate in the environment.

G.2.4 Determine the biomass in the test material (see Note) by taking samples of the inoculated medium at the beginning, before adding the test material, and of the test suspension at the end of the incubation period. Sampling must be carried out carefully to obtain representative samples. Filter the samples through a membrane filter or centrifuge at about 40 000 $m \cdot s^{-2}$ (see 3.5).

Determine the amount of biomass in the filtrate or centrifugate using a suitable method. Determine or assume the amount of carbon in the biomass and calculate, from the difference between the values at the beginning and end of the test, the increase in the amount of organic carbon in the biomass.

NOTE It may be difficult to calculate the exact amount of carbon in the biomass by indirect methods such as protein measurement, lipid or DNA measurement or measurement of metabolic activity. Due to the high biomass content of the samples, the determination of the biomass by indirect methods can lead to a high error in calculating the recovery rate. When using protein measurement, it is questionable to assume that the carbon content of the biomass is given by a fixed ratio of 1:1 (protein:carbon), because this ratio depends on physiological and nutritional factors. In fact, there is no satisfactory indirect method available for calculating the carbon content of the biomass.

G.2.5 Determine the amount of polymeric material remaining at the end of the test using all the remaining test suspensions. This is normally a difficult procedure but can be done either directly if a polymer-specific analysis is available or indirectly. In the first case, extract and measure the amount of residual polymeric material and calculate, from the known composition, the amount of carbon left. A possible indirect determination is washing, drying and weighing the residue and determining the total organic carbon (TOC). Then subtract the biomass carbon (obtained from the biomass determination) from the TOC to obtain the amount of carbon in the residual polymeric material.

G.3 Calculation of the recovery rate

Calculate the biochemically produced amounts of carbon (CO_2, IC and CH_4) in each test vessel (i.e. the amount of carbon gasified from the test material into the gas phase plus the amount dissolved in the medium) using Equations (G.1), (G.2) and (G.3):

$$C_{CO2} = (C_{CO2})_{T_1/T_2/T_3} - \text{Mean}(C_{CO2})_{B_1/B_2/B_3} \quad (G.1)$$

$$C_{CH4} = (C_{CH4})_{T_1/T_2/T_3} - \text{Mean}(C_{CH4})_{B_1/B_2/B_3} \quad (G.2)$$

$$C_{IC} = (C_{IC})_{T_1/T_2/T_3} - \text{Mean}(C_{IC})_{B_1/B_2/B_3} \quad (G.3)$$

where

C_{CO2}, C_{IC}, C_{CH4} are those parts of the carbon in the introduced test material which are biochemically converted into CO_2, IC and CH_4, expressed in mg;

$T_1/T_2/T_3$ indicate test vessels Nos. 1 to 3;

$B_1/B_2/B_3$ indicate blank vessels Nos. 1 to 3.

Calculate the increase in biomass carbon in each test vessel containing test material C_{BIO} by comparing the biomass at the beginning and the end of the incubation test period considering the measured or estimated amounts of carbon in the biomass, $C_{BIO(start)}$ and $C_{BIO(end)}$ in accordance with Equation (G.4):

$$C_{BIO} = C_{BIO(end)T_1/T_2/T_3} - C_{BIO(start)T_1/T_2/T_3} \quad (G.4)$$

where

C_{BIO} is that part of the carbon in the introduced test material which is biochemically converted into biomass, expressed in mg;

$C_{BIO(end)}$ is the carbon content of the biomass in the test vessels at the end of the test, expressed in mg.

$C_{BIO(start)}$ is the carbon content of the biomass in the test vessels at the beginning of the test, expressed in mg.

Determine the increase in DOC in each test vessel during the incubation period C_{DOC} by comparing the DOC concentration at the beginning $C_{DOC(start)}$ and the end $C_{DOC(end)}$ in accordance with Equation (G.5):

$$C_{DOC} = C_{DOC(end)T_1/T_2/T_3} - C_{DOC(start)T_1/T_2/T_3} \quad (G.5)$$

where

C_{DOC} is that part of the carbon in the test material introduced into the test vessels which is biochemically converted into DOC, expressed in mg;

$C_{DOC(end)}$ is the content of dissolved organic carbon in the test vessels at the end of the test, expressed in mg;

$C_{DOC(start)}$ is the content of dissolved organic carbon in the test vessels at the beginning of the test, expressed in mg.

Determine the amount of organic carbon in the polymeric material remaining in each test flask at the end of the test C_{POL}, expressed in mg.

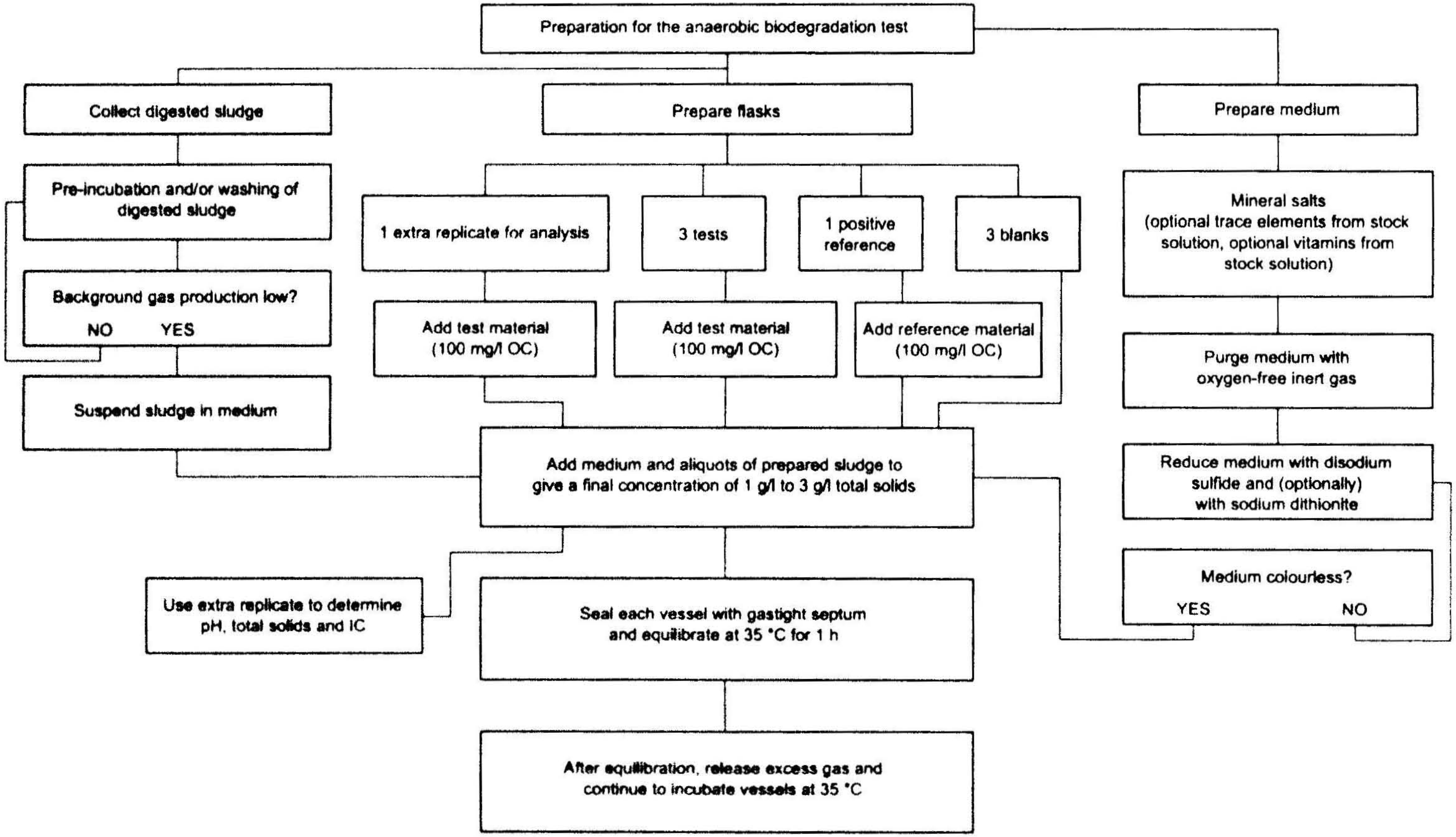
From the various amounts of converted carbon (C_{CO2}, C_{IC}, C_{CH4}, C_{BIO}, C_{DOC}, C_{POL}) calculated for the test vessels (T_1, T_2, T_3), calculate the recovery rate C_{REC}, expressed as a percentage of the total amount of carbon introduced C_{MAT}, from Equation (G.6):

$$C_{REC} = \frac{C_{CO2} + C_{IC} + C_{CH4} + C_{BIO} + C_{DOC} + C_{POL}}{C_{MAT}} \cdot 100 \qquad (G.6)$$

Annex H

(informative)

Example of a workflow scheme


Preparation for the anaerobic biodegradation test
Collect digested sludge
Prepare flasks
Prepare medium
Pre-incubation and/or washing of digested sludge
1 extra replicate for analysis
3 tests
1 positive reference
3 blanks
Mineral salts (optional trace elements from stock solution, optional vitamins from stock solution)
Background gas production low?
NO
YES
Add test material (100 mg/l OC)
Add test material (100 mg/l OC)
Add reference material (100 mg/l OC)
Purge medium with oxygen-free inert gas
Suspend sludge in medium
Add medium and aliquots of prepared sludge to give a final concentration of 1 g/l to 3 g/l total solids
Reduce medium with disodium sulfide and (optionally) with sodium dithionite
Medium colourless?
YES
NO
Use extra replicate to determine pH, total solids and IC
Seal each vessel with gastight septum and equilibrate at 35 °C for 1 h
After equilibration, release excess gas and continue to incubate vessels at 35 °C

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[9] ISO 11923, *Water quality — Determination of suspended solids by filtration through glass-fibre filters*

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